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Date: Oct. 21st, 2008

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TRANSLATION

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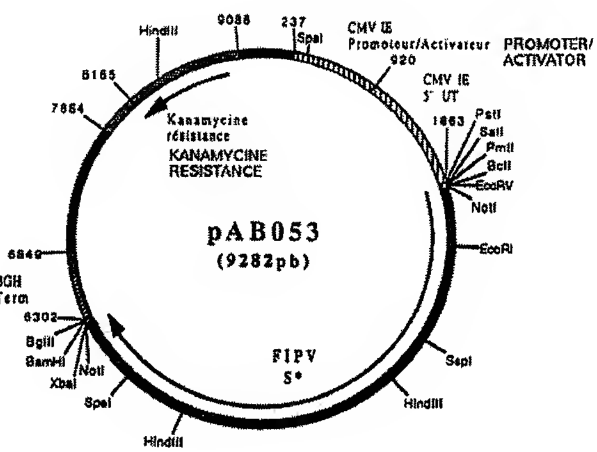
(54) Title: FELINE POLYNUCLEOTIDE VACCINE FORMULA

(54) Titre: FORMULE DE VACCIN POLYNUCLEOTIDIQUE FELIN

(57) Abstract

A cat vaccine formula including at least three polynucleotide vaccine valencies that each include a plasmid containing a cat pathogen valency gene capable of being expressed *in vivo* in host cells. Said valencies are selected from the group which consists of feline leukaemia virus, panleukopenia virus, infectious peritonitis virus, coryza virus, calicivirus disease virus, feline immunodeficiency virus and optionally rabies virus. Said plasmids include one or more genes per valency, and said genes are selected from the group which consists of env and gag for feline leukaemia, VP2 for panleukopenia, modified S, M and N for infectious peritonitis, gB and gD for coryza, capsid for calicivirus disease, env and gag/pro for feline immunodeficiency and G for rabies.



FELINE POLYNUCLEOTIDE VACCINE FORMULA

The present invention relates to a vaccine formula allowing the vaccination of cats against a number of pathologies. It also relates to a corresponding method of vaccination.

Associations of vaccines against certain canine viruses have already been proposed in the past.

The associations developed so far were prepared from inactivated vaccines or live vaccines and, optionally, from mixtures of such vaccines. Their development raises problems of compatibility between valencies and of stability. It is indeed necessary to ensure both the compatibility between the different vaccine valencies, whether from the point of view of the different antigens used or from the point of view of the formulations themselves, especially in the case where both inactivated vaccines and live vaccines are combined. The problem of the conservation of such combined vaccines and of their safety especially in the presence of an adjuvant also exists. These vaccines are in general quite expensive.

Patent Applications WO-A-90 11092, WO-A-93 19183, WO-A-94 21797 and WO-A-95 20660 have made use of the recently developed technique of polynucleotide vaccines. It is known that these vaccines use a plasmid capable of expressing, in the host cells, the antigen inserted into the plasmid. All routes of administration have been proposed (intraperitoneal, intravenous, intramuscular, transcutaneous, intradermal, mucosal and the like). Various vaccination means can also be used, such as DNA deposited at the surface of gold particles and projected so as to penetrate into the animals' skin (Tang et al., Nature 356, 152-154, 1992) and liquid jet injectors which make it possible to transfect at the same time the skin, the muscle, the fatty tissues and the mammary tissues (Furth et al., Analytical Biochemistry, 205, 365-368, 1992).

Polynucleotide vaccines may also use naked DNAs as well as DNAs formulated, for example, inside cationic lipids or liposomes.

The invention therefore proposes to provide a multivalent vaccine formula that makes possible to ensure vaccination against a number of feline pathogenic viruses.

Another objective of the invention is to provide such a vaccine formula combining different valencies while exhibiting all the criteria required for mutual compatibility and stability of the valencies.

Another objective of the invention is to provide such a vaccine formula which makes it possible to combine different valencies in the same vehicle.

Another objective of the invention is to provide such a vaccine which is easy and inexpensive to use.

Yet another objective of the invention is to provide a method for vaccinating cats allowing protection, including multivalent protection, with a high level of efficiency and of long duration, as well as good safety.

The subject of the present invention is therefore a vaccine formula intended for cats, comprising at least three polynucleotide vaccine valencies each comprising an integrating plasmid, so as to express it in vivo in the host cells, a gene of a feline pathogen valency, these valencies being selected from the group consisting of feline leukaemia virus (FeLV), panleukopenia virus (FPV), infectious peritonitis virus (FIPV), coryza virus (FHV), calicivirosis virus (FCV), feline immunodeficiency virus (FIV) and possibly rabies virus (rhabdovirus), the plasmids comprising, for each valency, one or more of the genes selected from the group consisting of env and gag/pol for the feline leukaemia, VP2 for the panleukopenia, modified S (or S*) and M for the infectious peritonitis, gB and gD for the coryza, capsid for the calicivirosis, env and gag/pro for the feline immunodeficiency and G for the rabies.

Valency in the present invention is understood to mean at least one antigen providing protection against the virus of the pathogen considered, wherein the valency may contain, as subvalency, one or more modified or natural genes from one or more strains of the pathogen considered.

Gene of a pathogenic agent is understood to mean not only the whole gene but also the various nucleotide sequences, including fragments which retain the ability to induce a protective response. The term gene encompasses nucleotide sequences equivalent to those precisely described in the examples, i.e. the sequences which are different but which encode the same protein. It also encompasses the nucleotide sequences of other strains of the pathogen

considered, providing a cross-protection or a protection specific for a strain or for a strain group. It also encompasses the nucleotide sequences which have been modified in order to facilitate the in vivo expression by the host animal but which encode the same protein.

Preferably, the vaccine formula according to the invention comprises the panleukopenia, coryza and calicivirosis valencies.

It will be possible to add the feline leukaemia, feline immunodeficiency and/or infectious peritonitis valencies.

As far as the coryza valency is concerned, it is preferable to use the two genes coding for gB and gD integrated in different plasmids or in a single plasmid, or to use either of these genes.

For the feline leukaemia valency, use is preferably made of the two env and gag/pol genes integrated into two different plasmids or into a single plasmid, or the env gene alone.

For the feline immunodeficiency valency, use will preferably be made of the two env and gag/pro genes in different plasmids or in a single plasmid, or only one of these genes. Still more preferably, the FeLV-A env gene and the FeLV-A and FeLV-B env genes are used.

For the infectious peritonitis valency, use is preferably made of the two M and modified S genes together in two different plasmids or in a single plasmid, or either of these genes. S will be modified in order to inactivate the major facilitating epitopes, preferably according to the teaching of Patent PCT/FR95/01128.

The vaccine formula according to the invention can be presented in a dose volume of between 0.1 and 3 ml and in particular between 0.5 and 1 ml.

The dose will be generally between 10 ng and 1 mg, preferably between 100 ng and 500 μg and still more preferably between 1 μg and 250 μg per plasmid type.

Use will preferably be made of naked plasmids simply placed in the vaccination vehicle which will be, in general, physiological saline (0.9% NaCl), ultrapure water, TE buffer and the like. All the polynucleotide vaccine forms described in the prior art can of course be used.

Each plasmid comprises a promoter capable of ensuring the expression into the host cells of the gene inserted, under its control. This will be in general a strong eukaryotic promoter

and in particular a cytomegalovirus early CMV-IE promoter of human or murine origin, or optionally of another origin such as rats, pigs and guinea pigs.

More generally, the promoter may be either of viral origin or of cellular origin. As viral promoter, there may be mentioned the SV40 virus early or late promoter or the Rous sarcoma virus LTR promoter. It may also be a promoter from the virus from which the gene is derived, for example the gene's own promoter.

As cellular promoter, there may be mentioned the promoter of a cytoskeleton gene, such as for example the desmin promoter (Bolmont et al., Journal of Submicroscopic Cytology and Pathology, 1990, 22, 117-122; and Zhenlin et al., Gene, 1989, 78, 243-254), or alternatively the actin promoter.

When several genes are present in the same plasmid, these may be presented in the same transcription unit or in two different units.

The combination of the different vaccine valencies according to the invention may preferably be achieved by mixing the polynucleotide plasmids expressing the antigen(s) of each valency, but it is also possible to have the same plasmid express antigens of several valencies.

The subject of the invention is also monovalent vaccine formulae comprising one or more plasmids encoding one or more genes from one of the viruses cited above, the genes being those described above. Besides their monovalent character, these formulae may possess the characteristics stated above regarding the choice of the genes, their combinations, the composition of the plasmids, the dose volumes, the doses and the like.

The monovalent vaccine formulae may also be used (i) for the preparation of a polyvalent vaccine formula as described above, (ii) individually against the actual pathology, (iii) combined with a vaccine of another type (live or inactivated whole, recombinant, subunit) against another pathology, or (iv) as booster for a vaccine as described below.

The subject of the present invention is in fact also the use of one or more plasmids according to the invention for the manufacture of a vaccine intended to vaccinate cats primo-vaccinated by means of a first conventional vaccine (monovalent or multivalent) as known in the prior art, in particular, selected from the group consisting of a live whole vaccine, an inactivated whole vaccine, a subunit vaccine, a recombinant vaccine, this first vaccine having (i.e.,

containing or capable of expressing) the antigen(s) encoded by the plasmid(s) or antigen(s) providing cross-protection.

Remarkably, the polynucleotide vaccine has a potent booster effect which results in an amplification of the immune response and the acquisition of a long-lasting immunity.

In general, the primo-vaccination vaccines can be selected from commercial vaccines available from various veterinary vaccine producers.

The subject of the invention is also a vaccination kit assembling together a primo-vaccination vaccine as described above and a vaccine formula according to the invention for the booster. It also relates to a vaccine formula according to the invention accompanied by a notice indicating the use of this formula as a booster for a primo-vaccination as described above.

The subject of the present invention is also a method for vaccinating cats, comprising the administration of an effective vaccine formula as described above. This vaccination method comprises the administration of one or more doses of the vaccine formula, wherein these doses can be administered successively over a short period of time and/or i successively at widely spaced intervals.

The vaccine formulae according to the invention can be administered in the context of this method of vaccination, by different routes of administration proposed in the prior art for polynucleotide vaccination and by means of known techniques of administration.

The subject of the invention is also a method of vaccination consisting in performing a primo-vaccination as described above and a booster with a vaccine formula according to the invention.

In a preferred embodiment of the process according to the invention, in a first instance, an animal is administered an effective dose of the vaccine of the conventional, especially inactivated, live, attenuated or recombinant, type, or alternatively a subunit vaccine, so as to provide a primo-vaccination, and, after a period preferably of 2 to 6 weeks, is administered the polyvalent or monovalent vaccine according to the invention.

The efficiency of presentation of the antigens to the immune system varies according to the tissues. In particular, the mucous membranes of the respiratory track serve as barriers to the entry of pathogens and are associated with lymphoid tissues which support local immunity.

Administration of a vaccine by contact with the mucous membranes, in particular the buccal mucous membrane, the pharyngeal mucous membrane and the mucous membrane of the bronchial region, is of great interest for the vaccination against respiratory and digestive pathologies.

Consequently, administration using the mucosal routes is a preferred mode of administration for the invention, using in particular nebulization or spray or drinking water. It will be possible to apply the vaccine formulae and the vaccination methods according to the invention in this context.

The invention also relates to a method of preparing the vaccine formulae, namely the preparation of the valencies and mixtures thereof, as evident from this description.

The invention will now be described in greater detail with the aid of the embodiments of the invention taken with reference to the accompanying drawings.

List of figures

Sequence listing SEQ ID No.

SEQ ID No. 1:	Oligonucleotide PB247
SEQ ID No. 2	Oligonucleotide PB249
SEQ ID No. 3:	Oligonucleotide PB281
SEQ ID No. 4:	Oligonucleotide PB282
SEQ ID No. 5:	Sequence of the FELV-B virus env gene
SEQ ID No. 6:	Oligonucleotide PB283
SEQ ID No. 7:	Oligonuclootide PB284
SEQ ID No. 8:	Sequence of the FeLV-A virus gag/pol gene (Glasgow-1 strain)
SEQ ID No. 9:	Oligonucleotide AB021
SEQ ID No. 10:	Oligonucleotide AB024
SEQ ID No. 11:	Oligonucleotide AB103
SEQ ID No. 12:	Oligonucleotide AB112
SEQ ID No. 13:	Oligonucleotide AB113
SEQ ID No. 14:	Oligonucleotide AB104
SEQ ID No. 15:	Oligonucleotide AB101
SEQ ID No. 16:	Oligonucleotide AB102
SEQ ID No. 17:	Oligonuclootide AB106
SEQ ID No. 18:	Oligonucleotide AB107
SEQ ID No. 19:	Oligonucleotide AB061
SEQ ID No. 20:	Oligonucleotide AB064
SEQ ID No. 21:	Oligonucleotide AB065
SEQ ID No. 22:	Oligonuclootide AB066
SEQ ID No. 23:	Oligonucleotide AB025
SEQ ID No. 24:	Oligonucleotide AB026
SEQ ID No. 25:	Oligonucleotide AB067
SEQ ID No. 26:	Oligonucleotide AB070
SEQ ID No. 27:	Oligonucleotide AB154
SEQ ID No. 28:	Oligonucleotide AB155
SEQ ID No. 29:	Oligonucleotide AB011
SEQ ID No. 30:	Oligonucleotide AB012

EXAMPLES

Example 1: Culture of the Viruses

The viruses are cultured on the appropriate cellular system until a cytopathic effect is obtained. The cellular systems to be used for each virus are well known to persons skilled in the art. Briefly, the cells sensitive to the virus used, cultured in Eagle's minimum essential medium (MEM medium) or another appropriate medium, are inoculated with the viral strain studied using a multiplicity of infection of 1. The infected cells are then incubated at 37°C. for a time necessary for the appearance of a complete cytopathic effect (on average 36 hours).

Example 2: Extraction of the Viral Genomic DNAs

After culture, the supernatant and the lysed cells are harvested and the entire viral suspension is centrifuged at 1000 g for 10 minutes at +4°C so as to remove the cellular debris. The viral particles are then harvested by ultracentrifugation at 400,000 g for 1 hour at +4°C. The pellet is taken up in a minimum volume of buffer (10 mM Tris, 1 mM EDTA). This concentrated viral suspension is treated with proteinase K (100 μg/ml final) in the presence of sodium dodecyl sulphate (SDS) (0.5% final) for 2 hours at 37°C. The viral DNA is then extracted with a mixture of phenol/chloroform and then precipitated with 2 volumes of absolute ethanol. After leaving the sample overnight at -20°C, the DNA is centrifuged at 10,000 g for 15 minutes at +4°C. The DNA pellet is dried and then taken up in a minimum volume of sterile ultrapure water. It can then be digested with restriction enzymes.

Example 3: Isolation of the Viral Genamic RNAs

The RNA viruses were purified according to techniques well known to one skilled in the art. The genomic viral RNA of each virus was then isolated using the "guanidium thiocyanate/phenol-chloroform" extraction technique described by P. Chomczynski and N. Sacchi (Anal. Biochem., 1987, 162, 156-159).

Example 4: Molecular Biology Techniques

All the constructions of plasmids were carried out using standard molecular biology techniques described by J. Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold

Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989). All the restriction fragments used for the present invention were isolated using the "Geneclean" kit (BIO 101 Inc. La Jolla, Calif.).

Example 5: RT-PCR Technique

Specific oligonucleotides (comprising restriction sites at their 5' ends to facilitate the cloning of the amplified fragments) were synthesized such that they completely cover the coding regions of the genes to be amplified (see specific examples). The reverse transcription (RT) reaction and polymerase chain reaction (PCR) were carried out according to standard techniques (Sambrook J. et al., 1989). Each RT-PCR reaction was performed with a pair of specific amplimers and using, as template, the extracted viral genomic RNA. The complementary DNA amplified was extracted with phenol/chloroform/isoamyl alcohol (25:24:1) before being digested with restriction enzymes.

Example 6: Plasmid pVR1012

The plasmid pVR1012 (Figure No. 1) was obtained from Vical Inc., San Diego, Calif., USA. Its construction has been described in J. Hartikka et al. (Human Gene Therapy, 1996, 7, 1205-1217).

Example 7: Construction of the Plasmid pPB179 (FeLV-A Virus Env Gene)

An RT-PCR reaction according to the technique described in Example 5 was carried out with feline leukaemia virus (FeLV-A) (Glasgow-1 strain) genomic RNA (M. Stewart et al. J. Virol. 1986. 58. 825-834), prepared according to the technique described in Example 3, and with the following oligonucleotides:

PB247 (29 mer) (SEQ ID No. 1)
5'TTTGTCGACCATGGAAAGTCCAACGCACC3'

PB249 (28 mer) (SEQ ID No. 2)
5'TTTGGATCCTCATGGTCGGTCCGGATCG3'

so as to amplify a 1947 bp fragment containing the gene encoding the Env glycoprotein from the FeLV-A virus (Glasgow-1 strain) in the form of a SalI-BamHI fragment. After purification, the RT-PCR product was digested with SalI and BamHI in order to give a 1935 bp SalI-BamHI fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with SalI and BamHI, to give the plasmid pPB179 (6804 bp) (Figure No. 2).

Example 8: Construction of the Plasmid pPB180 (FeLV-S Virus Env Gene)

An RT-PCR reaction according to the technique described in Example 5 was carried out with feline leukaemia virus (FeLV-B subtype) genomic RNA, prepared according to the technique described in Example 3, and with the following oligonucleotides:

PB281 (29 mer) (SEQ ID No. 3)

5'TTTGTCGACATGGAAGGTCCAACGCACCC3'

PB282 (32 mer) (SEQ ID No. 4)

5'TTGGATCCTCATGGTCGGTCCGGATCATATTG3'

so as to amplify a 2005 bp fragment containing the gene encoding the Env glycoprotein from the FeLV-B virus (Figure No. 3 and SEQ ID No. 5) in the form of a SalI-BamHI fragment. After purification, the RT-PCR product was digested with SalI and BamHI in order to give a 1995 bp SalI-BamHI fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with SalI and BamHI, to give the plasmid pPB180 (6863 bp) (Figure No. 4).

Example 9: Construction of the Plasmid pPB181 (FeLV gag/pol gene)

An RT-PCR reaction according to the technique described in Example 5 was carried out with the feline leukaemia virus (FeLV-A subtype) (Glasgow-1 strain) genomic RNA, prepared according to the technique described in Example 3, and with the following oligonucleotides:

PB283 (33 mer) (SEQ ID No. 6)

5'TTGTCGACATGTCTGGAGCCTCTAGTGGGACAG3'

PB284 (42 mer) (SEQ ID No. 7)

5'TTGGATCCTTATTTAATTACTGCAGTTCCAAGGAACTCTC3'

so as to amplify a 3049 bp fragment containing the sequence encoding the Gag protein and the 5' part of the sequence encoding the Pol protein from the FeLV-A virus (Glasgow-1 strain) (Figure

No. 5 and SEQ ID No. 8) in the form of a SalI-BamHI fragment. After purification, the RT-PCR product was digested with SalI and BamHI to give a 3039 bp SalI-BamHI fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with SalI and BamHI, to give the plasmid pPB181 (7908 bp) (Figure No. 6).

Example 10: Construction of the Plasmid pAB009 (FPV VP2 gene)

A PCR reaction was carried out with the feline panleukopaenia virus (193 strain) genomic DNA (J. Martyn et al., J. Gen. Virol. 1990, 71. 2747-2753), prepared according to the technique of Example 2, and with the following oligonucleotides:

AB021 (34 mer) (SEQ ID No. 9)
5'TGCTCTAGAGCAATGAGTGATGGAAGCAGTTCAAC3'

AB024 (33 mer) (SEQ ID No. 10)
5'CGCGGATCCATTAATATAATTTTCTAGGTGCTA3'

so as to amplify a 1776 bp fragment containing the gene encoding the FPV VP2 capsid protein. After purification, the PCR product was digested with XbaI and BamHI in order to give a 1764 bp XbaI-BamHI fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with XbaI and BamHI, to give the plasmid pAB009 (6664 bp) (Figure No. 7).

Example 11: Construction of the Plasmid pAB053 (FIPV S* gene)

An RT-PCR reaction according to the technique described in Example 5 was carried out with the feline infectious peritonitis (FIP) virus (79-1146 strain) genomic RNA (R. de Groot et al., J. Gen. Virol. 1987. 68. 2639-2646), prepared according to the technique described in Example 3, and with the following oligonucleotides:

AB103 (38 mer) (SEQ ID No. 11)
5'ATAAGAATGCGGCCGCATGATTGTGCTCGTAACTTGCC3'

AB112 (25 mer) (SEQ ID No. 12)
5'CGTACATGTGGAATTCCACTGGTTG3'

so as to amplify the sequence of the 5' part of the gene encoding the virus S glycoprotein in the form of an NotI-EcoRI fragment. After purification, the 492 bp RT-PCR product was digested with NotI and EcoRI in order to liberate a 467 bp NotI-EcoRI fragment (fragment A).

The plasmid pJCA089 (Patent Application PCT/FR95/01128) was digested with EcoRI and SpeI in order to liberate a 3378 bp fragment containing the central part of the gene encoding the FIP virus modified S glycoprotein (fragment B).

An RT-PCR reaction according to the technique described in Example 5 was carried out with the FIP virus (79-1146 strain) genomic RNA, prepared according to the technique described in Example 3, and with the following oligonucleotides:

AB113 (25 mer) (SEQ ID No. 13)
5'AGAGTTGCAACTAGTTCTGATTTTG3'

AB104 (37 mer) (SEQ ID No. 14)
5'ATAAGAATGCGGCCGCTTAGTGGACATGCACTTTTTC3'

so as to amplify the sequence of the 3' part of the gene encoding the FIP virus S glycoprotein in the form of an SpeI-NotI fragment. After purification, the 543 bp RT-PCR product was digested with SpeI and NotI in order to liberate a 519 bp SpeI-NotI fragment (fragment C).

Fragments A, B and C were then ligated together into the vector pVR1012 (Example 6), previously digested with NotI, to give the plasmid pAB053 (9282 bp), which contains the modified S gene in the correct orientation relative to the promoter (Figure No. 8).

Example 12: Construction of the Plasmid pAB052 (FIPV M gene)

An RT-PC'R reaction according to the technique described in Example 5 was carried out with the feline infectious peritonitis (FIP) virus (79-1146 strain) genomic RNA (H. Vennema et al., Virology. 1991, 181. 327-335), prepared according to the technique described in Example 3, and with the following oligonucleotides:

AB101 (37 mer) (SEQ ID No. 15)
5'ACGCGTCGACCCACCATGAAGTACATTTTGCTAATAC3'

AB102 (36 mer) (SEQ ID No. 16)
5'CGCGGATCCTTACACCATATGTAATAATTTTTCATG3'

so as to precisely isolate the gene encoding the FIP virus M glycoprotein in the form of a SalI-BamHI fragment. After purification, the 812 bp RT-PCR product was digested with SalI and BamHI in order to liberate a 799 bp SalI-BamHI fragment. This fragment was then ligated into the vector pVR1012 (Example 6), previously digested with SalI and BamHI, to give the plasmid pAB052 (5668 bp) (Figure No. 9).

Example 13: Construction of the Plasmid pAB056 (FIPV N gene)

An RT-PCP reaction according to the technique described in Example 5 was carried out with the feline infectious peritonitis (FIP) virus (79-1146 strain) genomic RNA (H. Vennema et ail., Virology. 1991, 181. 327-335), prepared according to the technique described in Example 3, and with the following oligonucleotides:

AB106 (35 mer) SEQ ID No. 17)

5'ACGCGTCGACGCCCTGGCCACACAGGGACAACGCG3'

AB107 (36 mer) (SEQ ID No. 18)

5'CGCGGATCCTTAGTCGTAACCTCATCAATCATCTC3'

so as to precisely isolate the gene encoding the FIP virus N protein in the form of a SalI-BamHI fragment. After purification, the 1156 bp RT-PCR product was digested with SalI and BamHI in order to liberate a 1143 bp SalI-BamHI fragment. This fragment was then ligated into the vector pVR1012 (Example 6), previously digested with SalI and BamHI, to give the plasmid pAB056 (6011 bp) (Figure No. 10).

Example 14: Construction of the Plasma pAB028 (FEV gB gene)

A PCR reaction was carried out with the feline herpesvirus (FHV-1) (C27 strain) genomic DNA (S. Spatz et al. Virology. 1993. 197. 125-36) prepared according to the technique of Example 2, and with the following oligonucleotides:

AB061 (36 mer) (SEQ ID No. 19)

5'AAAACTGCAGAATCATGTCCACTCGTGGCGATCTTG3'

AB064 (40 mer) (SEQ ID No. 20)

5'ATAAGAATGCGGCCCCTTAGACAAGATTTGTTTCAGTATC3'

so as to amplify a 2856 bp fragment containing the gene encoding the FHV-1 virus gB glycoprotein in the form of a PstI-NotI fragment. After purification, the PCR product was digested with PstI and NotI to give a 2823 bp PstI-NotI fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with PstI and NotI, to give the plasmid pAB028 (7720 bp) (Figure No. 11).

Example 15: Construction of the Plasmid pAB029 (FHV gD gene)

A PCR reaction was carried out with the feline herpesvirus (FHV-1) (C-27 strain) genomic DNA (S. Spatz et al. J. Gen. Virol. 1994. 75. 1235-1244), prepared according to the technique described in Example 2 and with the following oligonucleotides:

AB065 (36 mer) (SEQ ID No. 21)
5'AAAACTGCAGCCATGATGACACGTCTACATTTTG3'

AB066 (33 mer) (SEQ ID No. 22)
5'GGAAGATCTTTAAGGATGGTGAGTTGTATGTAT3'

so as to amplify the gene encoding the FHV-1 virus gD glycoprotein in the form of a PstI-BglII fragment. After purification, the 1147 bp PCR product was digested with PstI and BglII in order to isolate a 1129 bp PstI-BglII fragment. This fragment was ligated with the vector pVR1012 (Example 6), previously digested with PstI and BglII, to give the plasmid pAB029 (5982 bp) (Figure No. 12).

Example 16: Construction of the Plasmid pAB010 (FCV C gene)

An RT-PCR reaction according to the technique described in Example 5 was carried out with the feline calicivirus (FCV) (F9 strain) genomic RNA (M. Carter et al. Virology. 1992. 190. 443-448), prepared according to the technique described in Example 3, and with the following oligonucleotides:

AB025 (33 mer) (SEQ ID No. 23)
5'ACGCGTCGACGCATCTGCTCAACCTGCGCTAAC3'

AB026 (31 mer) (SEQ ID No. 24)
5'CGCGGATCCTCATAACTTAGTCATGGGACTC3'

so as to isolate the gene encoding the FCV virus capsid protein in the form of a SalI-BamHI fragment. After purification, the 2042 bp RT-PCR product was digested with SalI and BamHI in order to isolate a 2029 bp SalI-BamHI fragment. This fragment was ligated with the vector PVR1012 (Example 6), previously digested with SalI and BamHI, to give the plasmid pAB010 (6892 bp) (Figure No. 13).

Example 17: Construction of the Plasmid pAB030 (FIV env gene)

An RT-PCR reaction according to the technique of Example 5 was carried out with the feline immunodeficiency virus (FIV) (Petaluma strain) genomic RNA (R. Olmstec. et al. Proc. Natl. Acad. Sci. USA. 1989. 86. 8083-8096), prepared according to the technique of Example 3, and with the following oligonucleotides:

AB067 (36 mer) (SEQ ID No. 25)
5'AAAACTGCAGAAGGAATGGCAGAAGGATTTGCAGCC3'

AB070 (36 mer) (SEQ ID No. 26)
5'CGCGGATCCTCATTCCTCCTCTTTTTCAGACATGCC3'

so as to amplify a 2592 bp fragment containing the gene encoding the Env glycoprotein from the FIV virus (Petaluma strain) in the form of a PstI-BamHI fragment. After purification, the RT-PCR product was digested with PstI and BamHI to give a 2575 bp PstI-BamHI fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with PstI and BamHI, to give the plasmid pAB030 (7436 bp) (Figure No. 14).

Example 18: Construction of the Plasmid pAB083 (FIV gag/pro gene)

An RT-PCR reaction according to the technique described in Example 5 was carried out with the feline immunodeficiency virus (FIV) (Petaluma strain) genomic RNA (R. Olmsted et al. Proc. Natl. Acad. Sci. USA. 1989. 86. 8088-8096), prepared according to the technique described in Example 3, and with the following oligonucleotides:

AB154 (32 mer) (SEQ ID No. 27)
5'ACGCGTCGACATGGGGAATGGACAGGGGCGAG3'

AB155 (33 mer) (SEQ ID No. 28)

5'TGAAGATCTTCACTCATCCCCTTCAGGAAGAGC3'

so as to amplify a 4635 bp fragment containing the gene encoding the Gag and Pro proteins from the FIV virus (Petaluma strain) in the form of a SalI-BglII fragment. After purification, the RT-PCR product was digested with SalI and BglII to give a 4622 bp SalI-BglII fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with SalI and BglII, to give the plasmid pAB083 (7436 bp) (Figure No. 15).

Example 19: Construction of the Plasmid pAB041 (rabies virus G gene)

An RT-PC'R reaction according to the technique described in Example 5 was carried out with the rabies virus (ERA strain) genomic RNA (A. Anilionis et al. Nature. 1981. 294. 275-278), prepared according to the technique described in Example 3, and with the following oligonucleotides:

AB011 (33 mer) (SEQ ID No. 29)
5'AAAACTGCAGAGATGGTTCCTCAGGCTCTCCTG3'

AB012 (34 mer) (SEQ ID No. 30)
5'CGCGGATCCTCACACTCTGGTCTCACCCCCACTC3'

so as to amplify a 1589 bp fragment containing the gene encoding the rabies virus G glycoprotein. After purification, the RT-PCR product was digested with PstI and BamHI to give a 1578 bp PstI-BamHI fragment. This fragment was ligated with the vector pVR1012 (Example 6), previously digested with PstI and BamHI, to give the plasmid pAB041 (6437 bp) (Figure No. 16).

Example 20: Production and Purification of the Plasmids

For the preparation of the plasmids intended for the vaccination of animals, any technique may be used that allows for obtaining a suspension of purified plasmids predominantly in the supercoiled form. These techniques are well known to one skilled in the art. There may be mentioned in particular the alkaline lysis technique followed by two successive ultracentrifugations on a caesium chloride gradient in the presence of ethidium bromide as described in J. Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd edition, Cold

Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989). Reference may also be made to Patent Applications PCT WO 95/21250 and PCT WO 96/02658 which describe methods for producing, on an industrial scale, plasmids which can be used for vaccination. For the purposes of the manufacture of vaccines (see Example 17), the purified plasmids are resuspended so as to obtain solutions at a high concentration (>2 mg/ml) which are compatible with storage. To do so, the plasmids are resuspended either in ultrapure water or in TE buffer (10 mM Tris-HCl; 1 mM EDTA, pH 8.0).

Example 21: Manufacture of the Associated Vaccines

The various plasmids necessary for the manufacture of an associated vaccine are mixed using their concentrated solutions (Example 16). The mixtures are prepared such that the final concentration of each plasmid corresponds to the effective dose of each plasmid. The solutions which can be used to adjust the final concentration of the vaccine may be either a 0.9% NaCl solution, or PBS buffer.

Specific formulations such as liposomes, cationic lipids, may also be used for the manufacture of the vaccines.

Example 22: Vaccination of Cats

The cats are vaccinated with doses of 10 μg, 50 μg or 250 μg per plasmid.

The injections are performed with a needle using the intramuscular route. In that case, the vaccinal doses are administered in a volume of 1 ml.

The injections can also be performed with a needle using the intradermal route. In that case, the vaccinal doses are administered in a total volume of 1 ml administered in 10 injections of 0.1 ml or at 20 injections of 0.05 ml. The intradermal administrations are performed after shaving the skin (thoracic flank in general) or at the level of a relatively glabrous anatomical region, for example the inner surface of the thigh.

A liquid jet injection apparatus (with no needle) can also be used for the intradermal injections.

CLAIMS

1. Vaccine formula intended for cats, comprising at least three polynucleotide vaccine valencies each comprising an integrating plasmid, so as to express in vivo in the host cells, a gene of a feline pathogen valency, these valencies being selected from the group consisting of feline leukaemia virus, panleukopenia virus, infectious peritonitis virus, coryza virus, calicivirosis virus, feline immunodeficiency virus and possibly rabies virus, the plasmids comprising, for each valency, one or more of the genes selected from the group consisting of env and gag for the feline leukaemia, VP2 for the panleukopenia, modified S and M for the infectious peritonitis, gB and gD for the coryza, capsid for the calicivirosis, env and gag/pro for the feline immunodeficiency and G for the rabies.

2. Vaccine formula according to claim 1, characterized in that it comprises the panleukopenia, coryza and calicivirosis valencies.

3. Vaccine formula according to claim 1 or 2, characterized in that it comprises the coryza virus gB and gD genes, in the same plasmid or in different plasmids, or only one of these genes.

4. Formula according to claim 1, characterized in that it comprises the feline leukaemia virus env and gag genes, in the same plasmid or indifferent plasmids, or the env gene alone.

5. Vaccine formula according to claim 1, characterized in that it comprises the two env and gag/pro genes in different plasmids or in the same plasmid, or only one of these genes.

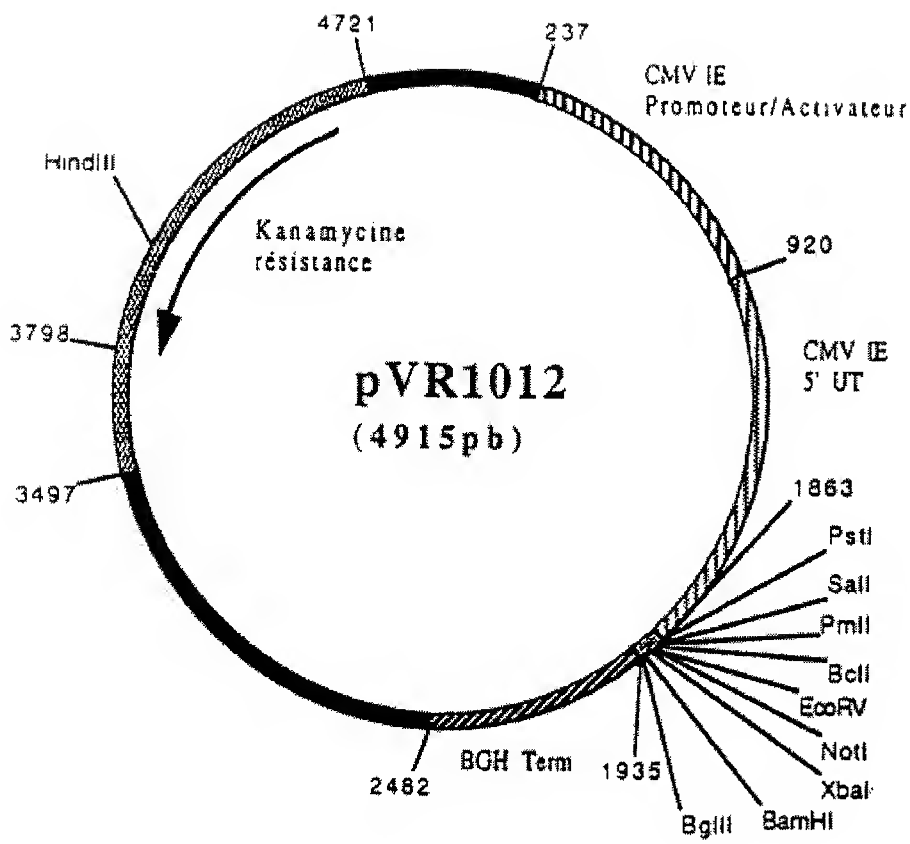
6. Vaccine formula according to claim 1 or 2, characterized in that it comprises the M gene or the modified S gene in a plasmid or all the genes encoding M or modified S in the same plasmid or in different plasmids.

7. Vaccine formula according to any one of claims 1 to 6, characterized in that it comprises from 10 ng to 1 mg, preferably from 100 ng to 500 μg, still more preferably from 1 μg to 250 μg of each plasmid.

8. Use of one or more plasmids as described in any one of claims 1 to 7, for the manufacture of a vaccine intended to vaccinate cats primo-vaccinated by means of a first vaccine selected from the group consisting of a live whole vaccine, an inactivated whole vaccine, a subunit vaccine, a recombinant vaccine, the first vaccine having the antigen(s) encoded by the plasmid(s) or antigen(s) providing cross-protection.

9. Vaccination kit for cats assembling together a vaccine formula according to any one of claims 1 to 8 and a vaccine for cats selected from the group consisting of a live whole vaccine, an inactivated whole vaccine, a subunit vaccine, a recombinant vaccine, the first vaccine having the antigen encoded by the polynucleotide vaccine or an antigen providing cross-protection, for administration of the latter in primo-vaccination and as a booster with the vaccine formula.

10. Vaccine formula according to any one of claims 1 to 8, accompanied by a notice indicating that this formula can be used as booster for a first vaccine for cats, selected from the group consisting of a live whole vaccine, an inactivated whole vaccine, a subunit vaccine, a recombinant vaccine, this first vaccine having the antigen encoded by the polynucleotide vaccine or an antigen providing cross-protection.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Promoteur/Activateur
HindIII
Kanamycine
résistance
920
3798
CMV IE
5' UT
pVR1012
(4915pb)
3497
1863
PstI
SalI
PmlI
BclI
EcoRV
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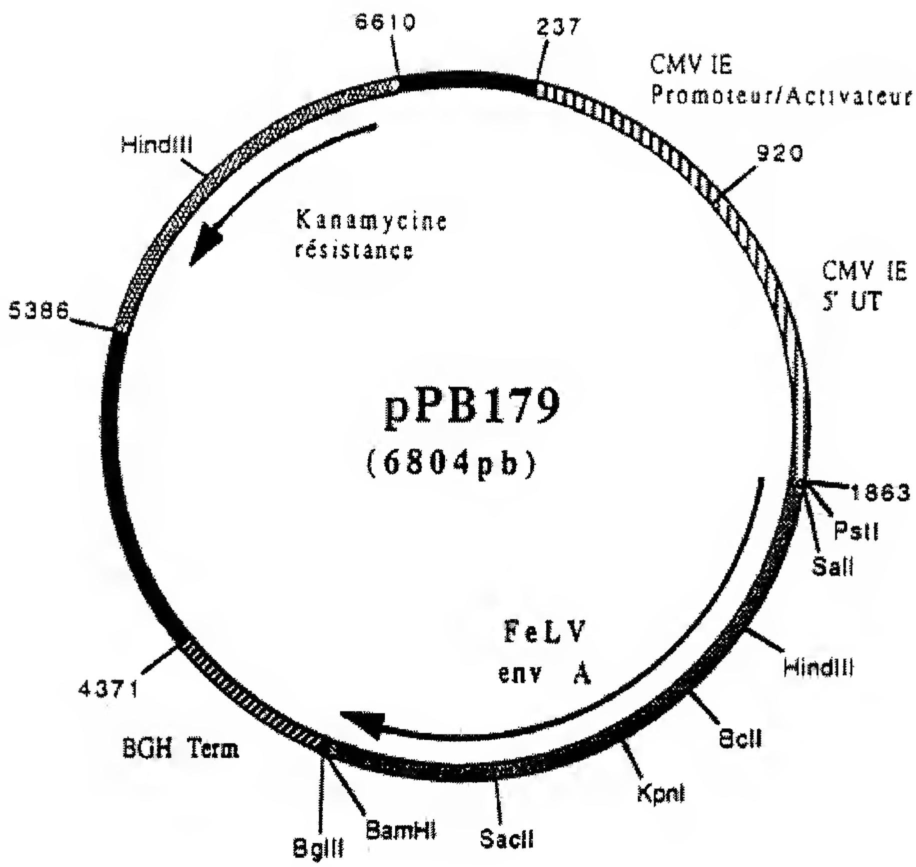
XbaI
BGH Term
1935
2482
BglII
BamHI

Figure No. 1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CMV IE
Promoteur/Activateur
HindIII
920
Kanamycine
résistance
CMV IE
5' UT
5386
pPB179
(6804pb)
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PstI
SalI
FeLV
env A
HindIII
4371
BclI
BGH Term
KpnI
BglII
BamHI
SacII

Figure No. 2

```
1    ATGGAAGGTCCAACGCACCCAAAACCCTCTAAAGATAAGACTTTCTCGTGGGACCTAATGATT
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64   CTGGTGGGGGTCTTACTAAGACTGGACGTGGGAATGGCCAATCCTAGTCCGCACCAAATATAT
22▶  LeuValGlyValLeuLeuArgLeuAspValGlyMetAlaAsnProSerProHisGlnIleTyr

127  AATGTAACTTGGACAATAACCAACCTTGTAACTGGAACAAAGGCTAATGCCACCTCCATGTTG
43▶  AsnValThrTrpThrIleThrAsnLeuValThrGlyThrLysAlaAsnAlaThrSerMetLeu

190  GGAACCCTGACAGACGCCTTCCCTACCATGTATTTTGACTTATGTGATATAATAGGAAATACA
64▶  GlyThrLeuThrAspAlaPheProThrMetTyrPheAspLeuCysAspIleIleGlyAsnThr

253  TGGAACCCTTCAGATCAAGAACCATTCCCAGGGTATGGATGTGATCAGCCTATGAGGAGGTGG
85▶  TrpAsnProSerAspGlnGluProPheProGlyTyrGlyCysAspGlnProMetArgArgTrp

316  CGACAGAGAAACACACCCTTTTATGTCTGTCCAGGACATGCCAACCGGAAGCAATGTGGGGGG
106▶ ArgGlnArgAsnThrProPheTyrValCysProGlyHisAlaAsnArgLysGlnCysGlyGly

379  CCACAGGATGGGTTCTGCGCTGTATGGGGTTGCGAGACCACCGGGGAAACCTATTGGAGACCC
127▶ ProGlnAspGlyPheCysAlaValTrpGlyCysGluThrThrGlyGluThrTyrTrpArgPro

442  ACCTCCTCATGGGACTACATCACAGTAAAAAAAGGGGTTACTCAGGGAATATATCAATGTAGT
148▶ ThrSerSerTrpAspTyrIleThrValLysLysGlyValThrGlnGlyIleTyrGlnCysSer

505  GGAGGTGGTTGGTGTGGGCCCTGTTACGATAAAGCTGTTCACTCCTCGACAACGGGAGCTAGT
169▶ GlyGlyGlyTrpCysGlyProCysTyrAspLysAlaValHisSerSerThrThrGlyAlaSer

568  GAAGGGGGCCGGTGCAACCCCTTGATCTTGCAATTTACCCAAAAGGGAAGACAAACATCTTGG
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631  GATGGACCTAAGTCATGGGGGCTACGACTATACCGTTCAGGATATGACCCTATAGCCCTGTTC
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694  TCGGTATCCCGGCAAGTAATGACCATTACGCCGCCTCAGGCCATGGGACCAAATCTAGTCCTG
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883  CCTGTCACCCCCGCAAGTCCCAAACGTATAGGGACCGGAAATAGGTTAATAAATTTAGTGCAA
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946  GGGACATACCTAGCCTTAAATGCCACCGACCCCAACAAAACTAAAGACTGTTGGCTCTGCCTG
316▶ GlyThrTyrLeuAlaLeuAsnAlaThrAspProAsnLysThrLysAspCysTrpLeuCysLeu

1009 GTTTCTCGACCACCTTATTACGAAGGGATTGCAATCTTAGGTAACTACAGCAACCAAACAAAC
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1072 CCCTCCCCATCCTGCCTATCTACTCCGCAACATAAGCTAACTATATCTGAGGTGTCAGGGCAA
358▶ ProSerProSerCysLeuSerThrProGlnHisLysLeuThrIleSerGluValSerGlyGln

1135 GGACTGTGCATAGGGACTGTTCCTAAGACCCACCAGGCTTTGTGCAATAAGACACAACAGGGA
379▶ GlyLeuCysIleGlyThrValProLysThrHisGlnAlaLeuCysAsnLysThrGlnGlnGly

1198 CATACAGGGGCTCACTATCTAGCCGCCCCCAATGGCACCTATTGGGCCTGTAACACTGGACTC
400▶ HisThrGlyAlaHisTyrLeuAlaAlaProAsnGlyThrTyrTrpAlaCysAsnThrGlyLeu
```

Figure No. 3

```
1261  ACCCCATGCATTTCCATGGCAGTGCTCAATTGGACCTCTGATTTTTGTGTCTTAATCGAATTA
 421▸ ThrProCysIleSerMetAlaValLeuAsnTrpThrSerAspPheCysValLeuIleGluLeu

1324  TGGCCCAGAGTGACCTACCATCAACCCGAATACATTTACACACATTTCGACAAAGCTGTCAGG
 442▸ TrpProArgValThrTyrHisGlnProGluTyrIleTyrThrHisPheAspLysAlaValArg

1387  TTCCGAAGAGAACCAATATCACTAACCGTTGCCCTTATAATGGGAGGACTCACTGTAGGGGGC
 463▸ PheArgArgGluProIleSerLeuThrValAlaLeuIleMetGlyGlyLeuThrValGlyGly

1450  ATAGCCGCGGGGGTCGGAACAGGGACTAAAGCCCTCCTTGAAACAGCCCAGTTCAGACAACTA
 484▸ IleAlaAlaGlyValGlyThrGlyThrLysAlaLeuLeuGluThrAlaGlnPheArgGlnLeu

1513  CAAATGGCTATGCACGCAGACATCCAGGCCCTAGAAGAGTCAATTAGTGCCTTAGAAAAATCC
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1639  AAGGGAGGGCTCTGTGCCGCCTTAAAGGAAGAATGCTGCTTCTATGCAGATCACACCGGACTC
 547▸ LysGlyGlyLeuCysAlaAlaLeuLysGluGluCysCysPheTyrAlaAspHisThrGlyLeu

1702  GTCAGAGACAATATGGCTAAATTAAGAGAAAGACTGAAACAGCGACAACAACTGTTTGACTCC
 568▸ ValArgAspAsnMetAlaLysLeuArgGluArgLeuLysGlnArgGlnGlnLeuPheAspSer

1765  CAACAGGGATGGTTTGAAGGATGGTTCAACAAGTCCCCCTGGTTTACAACCCTAATTTCCTCC
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1891  TTAGTGCAATTCGTAAAAGACAGAATATCTGTGGTACAAGCCTTAATTTTAACCCAACAGTAC
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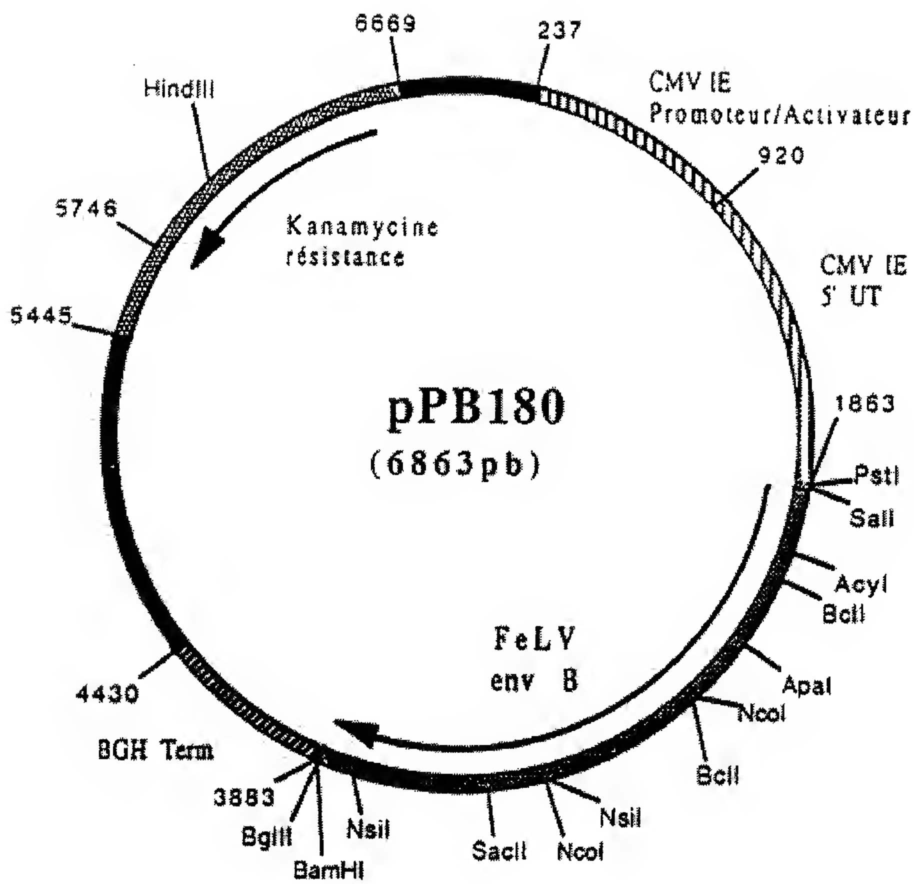
Figure No. 3 (last part)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PstI
SalI
AcyI
BclI
FeLV
env B
ApaI
4430
NcoI
BclI
BGH Term
3883
NsiI
BglII
NsiI
SacII
NcoI
BamHI

Figure No. 4

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1▸ MetSerGlyAlaSerSerGlyThrAlaIleGlyAlaHisLeuPheGlyValSerProGluTyr

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22▸ ArgValLeuIleGlyAspGluGlyAlaGlyProSerArgSerLeuSerGluValSerPheSer

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43▸ ValTrpTyrGlnArgArgAlaAlaArgLeuValIlePheCysLeuValAlaSerPheLeuVal

190 CCTTGTCTAACCTTTTTAATTGCAGAAACCGTCATGGGCCAAACTATAACTACCCCCTTAAGC
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106▸ LysLysLysTrpIleThrLeuCysGluAlaGluTrpValMetMetAsnValGlyTrpProArg

379 GAAGGAACTTTTTCTCTTGATAGCATTTCCCAGGTTGAAAAGAAGATCTTCGCCCCGGGACCA
127▸ GluGlyThrPheSerLeuAspSerIleSerGlnValGluLysLysIlePheAlaProGlyPro

442 TATGGACACCCCGACCAAGTTCCTTACATTACTACATGGAGATCCTTAGCCACAGACCCCCCT
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Figure No. 5

```
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1261  CCAGCAGCATTTTTAGAAAGATTAAAAGAGGCTTATAGAATGTACACTCCCTATGACCCTGAG
 421▸ ProAlaAlaPheLeuGluArgLeuLysGluAlaTyrArgMetTyrThrProTyrAspProGlu

1324  GACCCAGGGCAAGCGGCTAGTGTTATCCTATCCTTTATATACCAGTCTAGCCCAGATATAAGA
 442▸ AspProGlyGlnAlaAlaSerValIleLeuSerPheIleTyrGlnSerSerProAspIleArg

1387  AATAAGTTACAAAGGCTAGAAGGCCTACAAGGGTTCACCCTATCTGATCTGCTAAAAGAGGCA
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1450  GAAAAGATATACAACAAAAGGGAGACCCCAGAGGAAAGGGAAGAAAGATTATGGCAGCGACAG
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1513  GAAGAAAGAGATAAAAAGCGCCACAAGGAGATGACTAAAGTTCTGGCCACAGTAGTTGCTCAG
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1576  AATAGAGATAAGGATAGAGAAGAAAGTAAACTGGGGGATCAAAGGAAAATACCTCTGGGGAAA
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                                       1▸ GluIleArgArgValArgAlaArgThrPr

1765  CCCCCCTGAGCCCAGGATAACCTTAAAAATAGGGGGGCAACCGGTGACTTTTCTGGTGGAC

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  31▸ rGlyAlaGlnHisSerValLeuThrArgProAspGlyProLeuSerAspArgThrAlaLeuVa

1891  GCAAGGAGCCACGGGAAGCAAAAACTACCGGTGGACCACCGACAGGAGGGTACAACTGGCAAC

  52▸ lGlnGlyAlaThrGlySerLysAsnTyrArgTrpThrThrAspArgArgValGlnLeuAlaTh

1954  CGGTAAGGTGACTCATTCTTTTTATATGTACCTGAATGTCCCTACCCGTTATTAGGGAGAGA

  73▸ rGlyLysValThrHisSerPheLeuTyrValProGluCysProTyrProLeuLeuGlyArgAs

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  94▸ pLeuLeuThrLysLeuLysAlaGlnIleHisPheThrGlyGluGlyAlaAsnValValGlyPr

2080  CAGGGGTTTACCCCTACAAGTCCTTACTTTACAATTAGAAGAGGAGTATCGGCTATTTGAGCC

 115▸ oArgGlyLeuProLeuGlnValLeuThrLeuGlnLeuGluGluGluTyrArgLeuPheGluPr

2143  AGAAAGTACACAAAAACAGGAGATGGACACTTGGCTTAAAAACTTTCCCCAGGCGTGGGCAGA

 136▸ oGluSerThrGlnLysGlnGluMetAspThrTrpLeuLysAsnPheProGlnAlaTrpAlaGl
```

Figure No. 5 (middle part)

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157▶ uThrGlyGlyMetGlyMetAlaHisCysGlnAlaProValLeuIleGlnLeuLysAlaThrAl

2269 CACTCCAATCTCCATCCGACAGTATCCTATGCCCCATGAAGCGTACCAGGGAATTAAGCCTCA

178▶ aThrProIleSerIleArgGlnTyrProMetProHisGluAlaTyrGlnGlyIleLysProHi

2332 TATAAGAAGAATGCTAGATCAAGGCATCCTCAAGCCCTGCCAGTCCCCATGGAATACACCCTT

199▶ sIleArgArgMetLeuAspGlnGlyIleLeuLysProCysGlnSerProTrpAsnThrProLe

2395 ATTACCTGTTAAGAAGCCAGGGACCGAGGATTACAGACCAGTGCAGGACTTAAGAGAAGTAAA

220▶ uLeuProValLysLysProGlyThrGluAspTyrArgProValGlnAspLeuArgGluValAs

2458 CAAAAGAGTAGAAGACATCCATCCTACTGTGCCAAATCCATATAACCTCCTTAGCACCCTCCC

241▶ nLysArgValGluAspIleHisProThrValProAsnProTyrAsnLeuLeuSerThrLeuPr

2521 GCCGTCTCACCCTTGGTACACTGTCCTAGATTTAAAGGACGCTTTTTCTGCCTGCGACTACA

262▶ oProSerHisProTrpTyrThrValLeuAspLeuLysAspAlaPhePheCysLeuArgLeuHi

2584 CTCTGAGAGTCAGTTACTTTTTGCATTTGAATGGAGAGATCCAGAAATAGGACTGTCAGGGCA

283▶ sSerGluSerGlnLeuLeuPheAlaPheGluTrpArgAspProGluIleGlyLeuSerGlyGl

2647 ACTAACCTGGACACGCCTTCCTCAGGGGTTCAAGAATAGCCCCACCCTATTTGATGAGGCCCT

304▶ nLeuThrTrpThrArgLeuProGlnGlyPheLysAsnSerProThrLeuPheAspGluAlaLe

2710 GCACTCAGACCTGGCCGATTTCAGGGTAAGGTACCCGGCTCTAGTCCTCCTACAATATGTAGA

325▶ uHisSerAspLeuAlaAspPheArgValArgTyrProAlaLeuValLeuLeuGlnTyrValAs

2773 TGACCTCTTGCTGGCTGCGGCAACCAGGACTGAATGCCTGGAAGGGACTAAGGCACTCCTTGA

346▶ pAspLeuLeuLeuAlaAlaAlaThrArgThrGluCysLeuGluGlyThrLysAlaLeuLeuGl

2836 GACTTTGGGCAATAAGGGGTACCGAGCCTCTGGAAAGAAGGCCCAAATTTGCCTGCAAGAAGT

367▶ uThrLeuGlyAsnLysGlyTyrArgAlaSerGlyLysLysAlaGlnIleCysLeuGlnGluVa

2899 CACATACCTGGGGTACTCTTTAAAAGATGGCCAAAGGTGGCTTACCAAAGCTCGGAAAGAAGC

388▶ lThrTyrLeuGlyTyrSerLeuLysAspGlyGlnArgTrpLeuThrLysAlaArgLysGluAl

2962 CATCCTATCCATCCCTGTGCCTAAAAACCCACGACAAGTGAGAGAGTTCCTTGGAACTGCAG

409▶ aIleLeuSerIleProValProLysAsnProArgGlnValArgGluPheLeuGlyThrAla

Figure No. 5 (Last part)

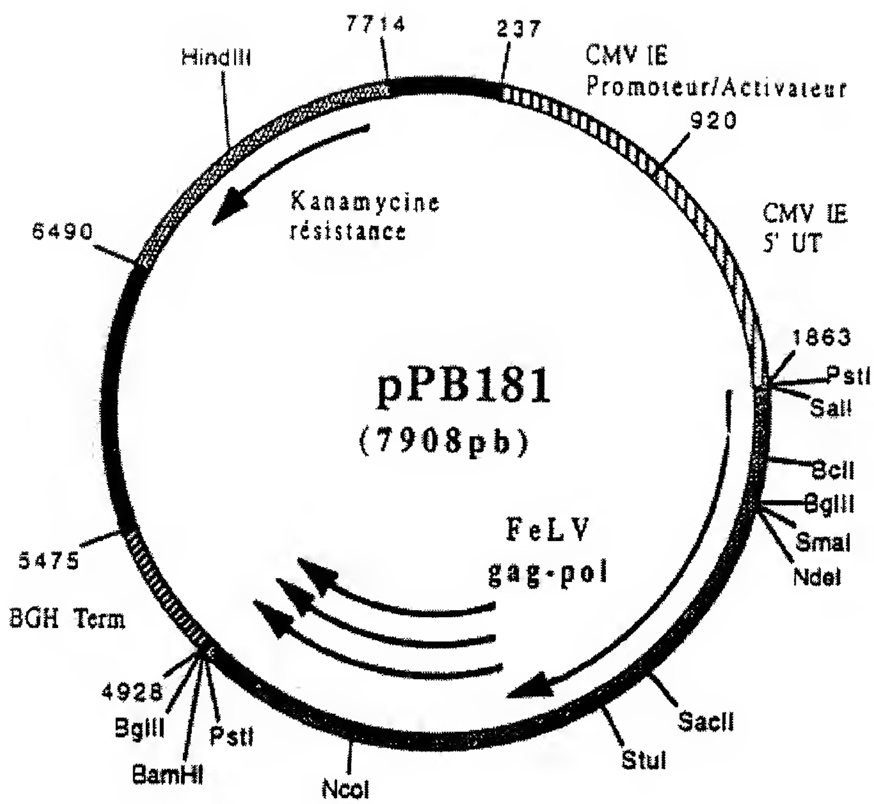

pPB181
(7908pb)
7714
237
HindIII
CMV IE
Promoteur/Activateur
920
Kanamycine
résistance
CMV IE
5' UT
6490
1863
PstI
SalI
BclI
BglII
SmaI
NdeI
FeLV
gag-pol
5475
BGH Term
4928
BglII
PstI
BamHI
NcoI
StuI
SacII

Figure No. 6

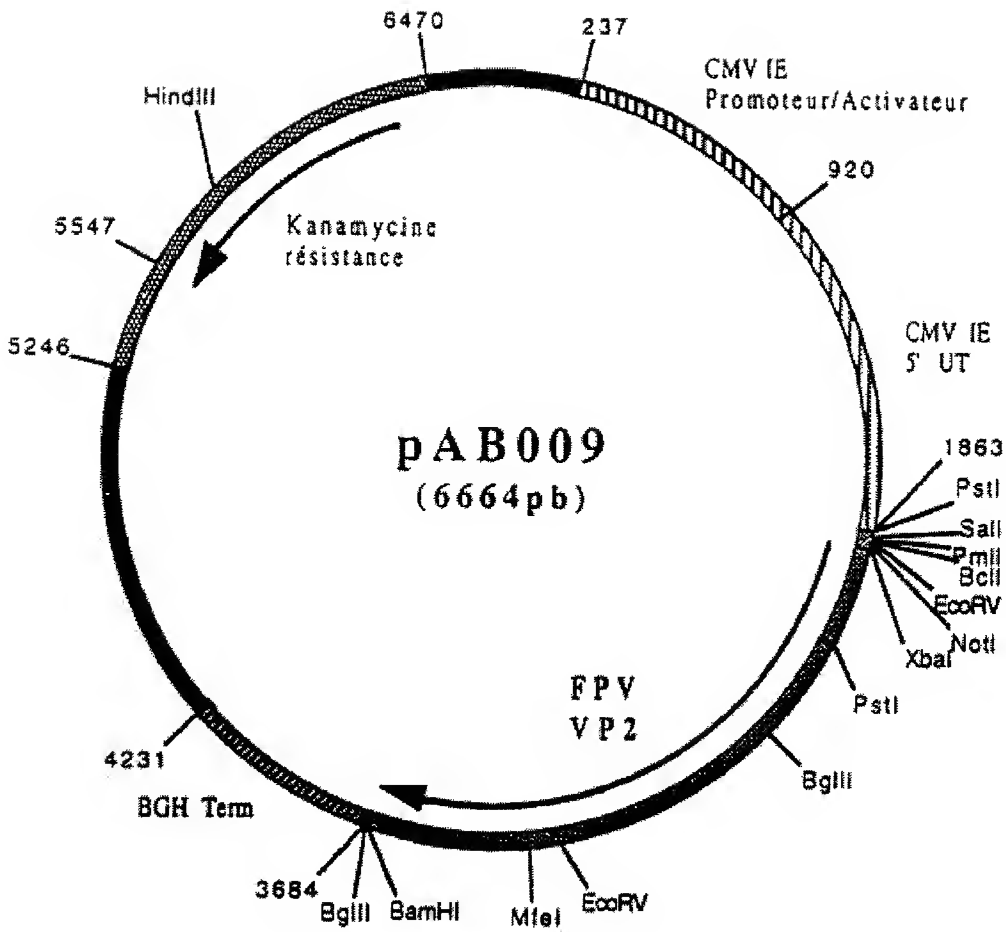
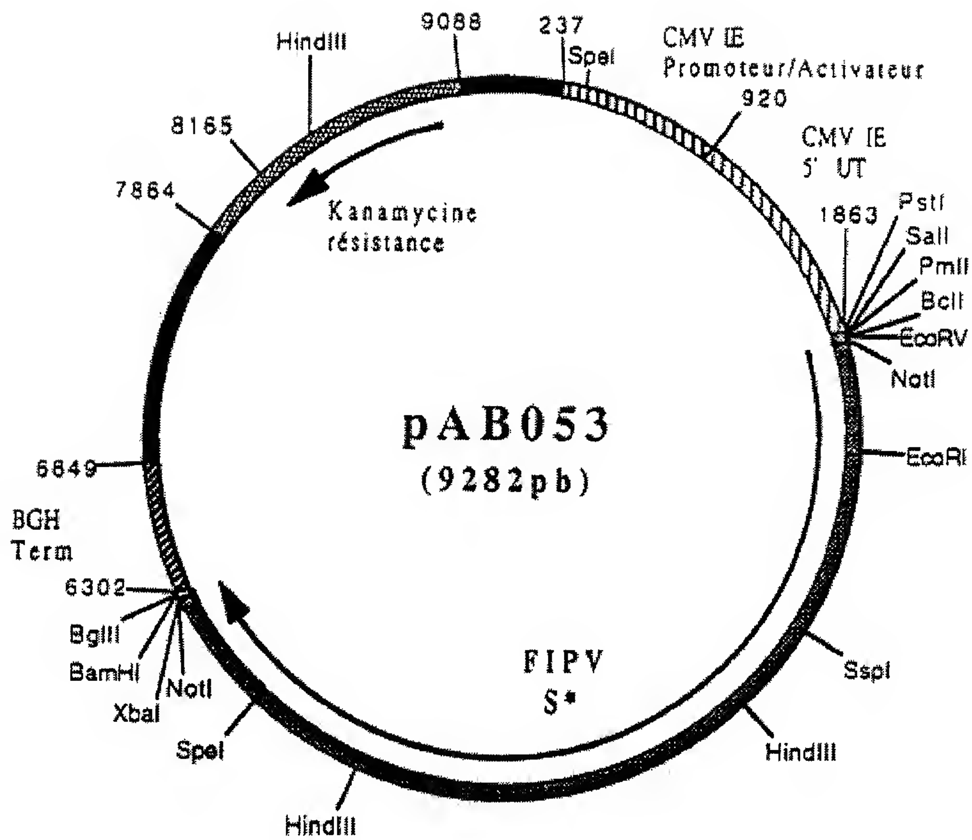

6470
237
CMV IE
Promoteur/Activateur
HindIII
920
5547
Kanamycine
résistance
5246
CMV IE
5' UT
pAB009
(6664pb)
1863
PstI
SalI
PmlI
BclI
EcoRV
NotI
XbaI
PstI
FPV
VP2
BglII
4231
BGH Term
3684
BglII
BamHI
MfeI
EcoRV

Figure No. 7



9088
237
SpeI
CMV IE
Promoteur/Activateur
920
CMV IE
5' UT
1863
PstI
SalI
PmlI
BclI
EcoRV
NotI
EcoRI
SspI
HindIII
HindIII
SpeI
XbaI
NotI
BamHI
BglII
6302
BGH
Term
6849
7864
8165
HindIII
Kanamycine
résistance
pAB053
(9282pb)
FIPV
S*

Figure No. 8

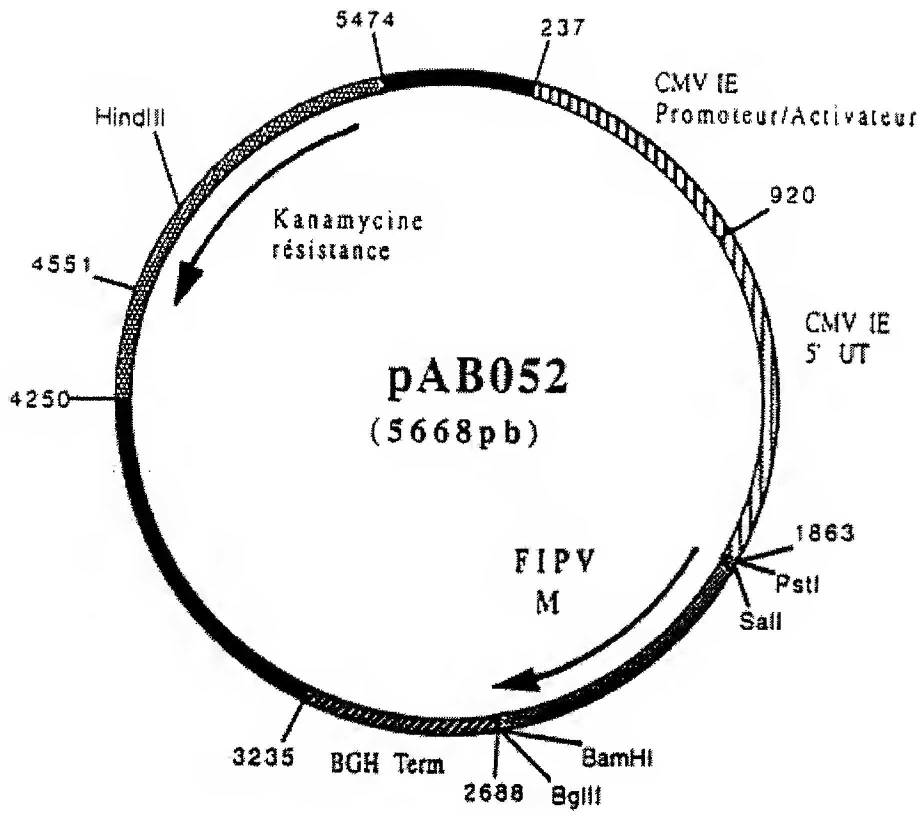

5474
237
CMV IE
Promoteur/Activateur
HindIII
920
Kanamycine
résistance
4551
CMV IE
5' UT
pAB052
(5668pb)
4250
1863
FIPV
M
PstI
SalI
3235
BGH Term
BamHI
2688
BglII

Figure No. 9

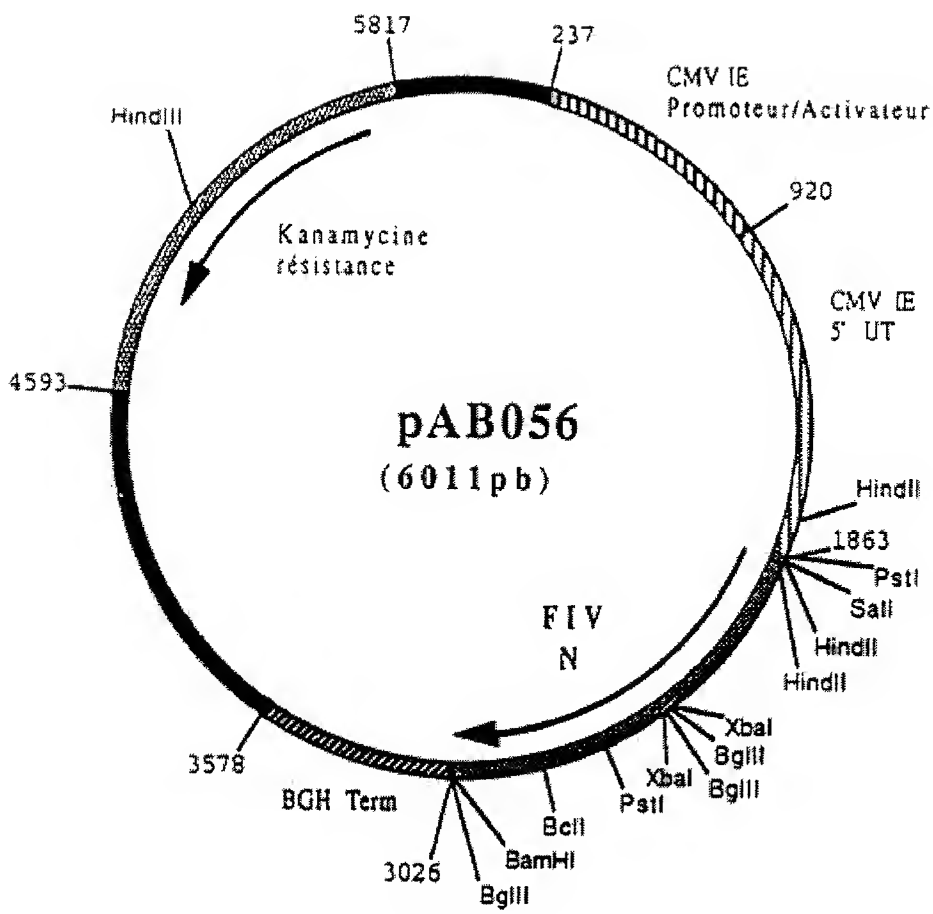

5817
237
CMV IE
Promoteur/Activateur
HindIII
920
Kanamycine
résistance
CMV IE
5' UT
4593
pAB056
(6011pb)
HindII
1863
PstI
SalI
HindII
HindII
FIV
N
XbaI
BglII
BglII
XbaI
PstI
3578
BGH Term
BclI
BamHI
3026
BglII

Figure No. 10

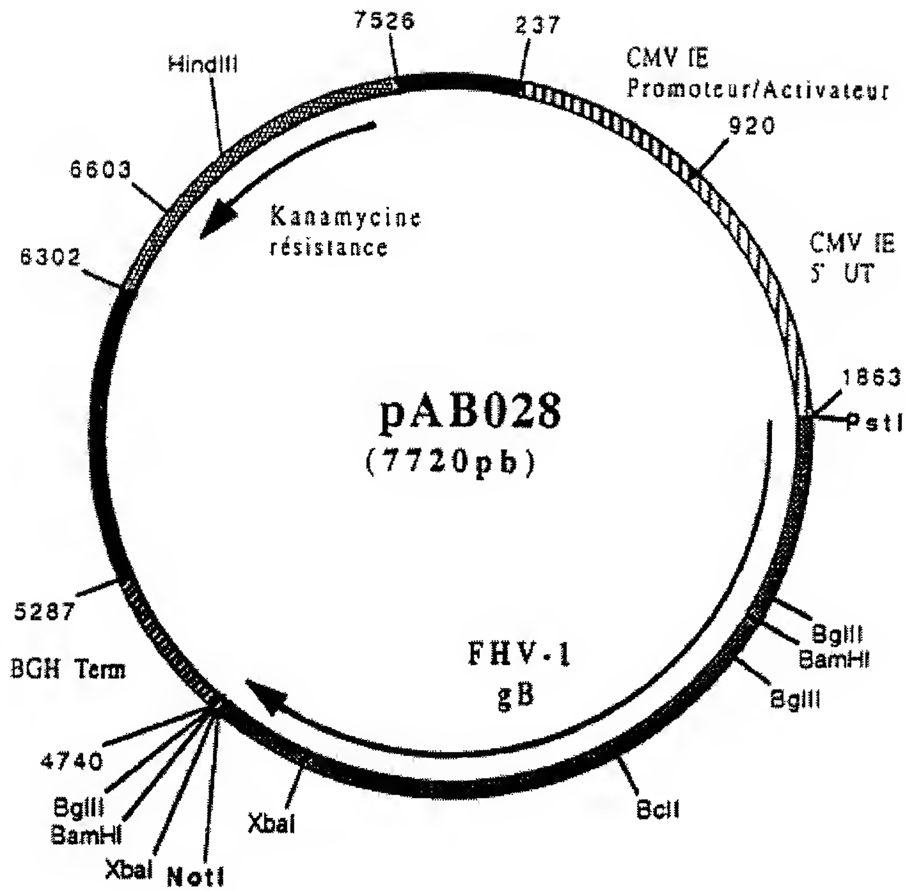

7526
237
HindIII
CMV IE
Promoteur/Activateur
920
6603
Kanamycine
résistance
6302
CMV IE
5' UT
1863
PstI
pAB028
(7720pb)
5287
BglII
BamHI
BGH Term
FHV-1
gB
BglII
4740
BglII
BamHI
XbaI
BclI
XbaI
NotI

Figure No. 11

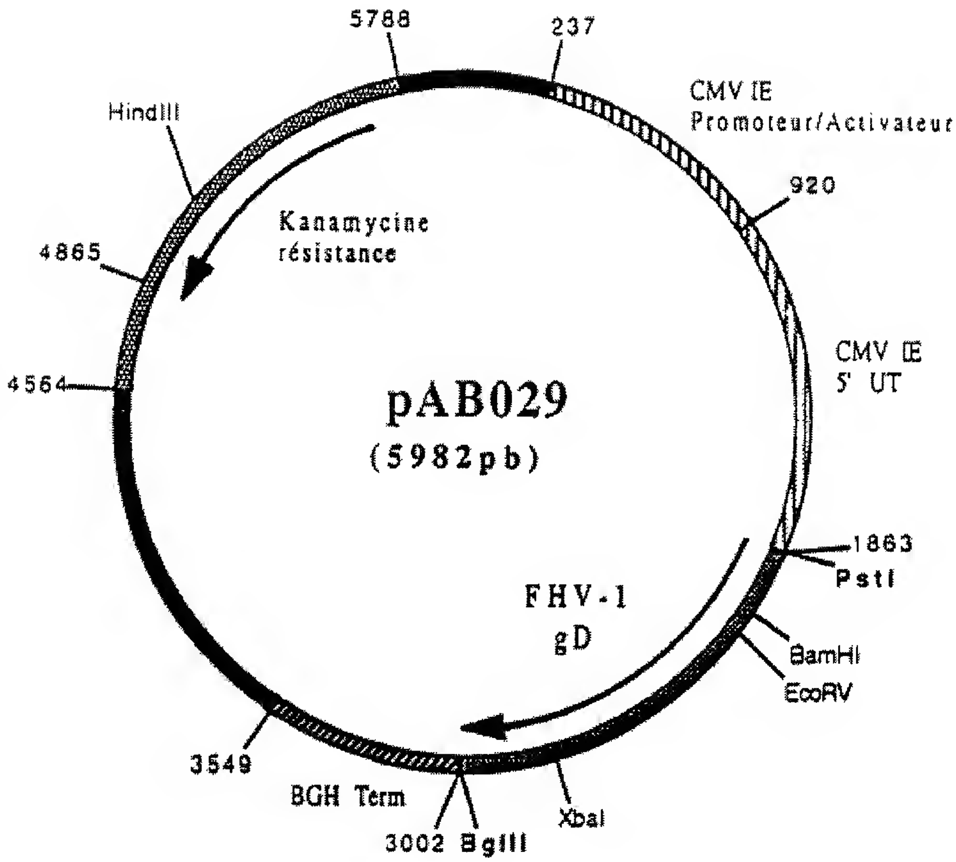

5788
237
CMV IE
Promoteur/Activateur
HindIII
920
Kanamycine
résistance
4865
CMV IE
5' UT
4564
pAB029
(5982pb)
1863
PstI
FHV-1
gD
BamHI
EcoRV
3549
BGH Term
XbaI
3002 BglII

Figure No. 12

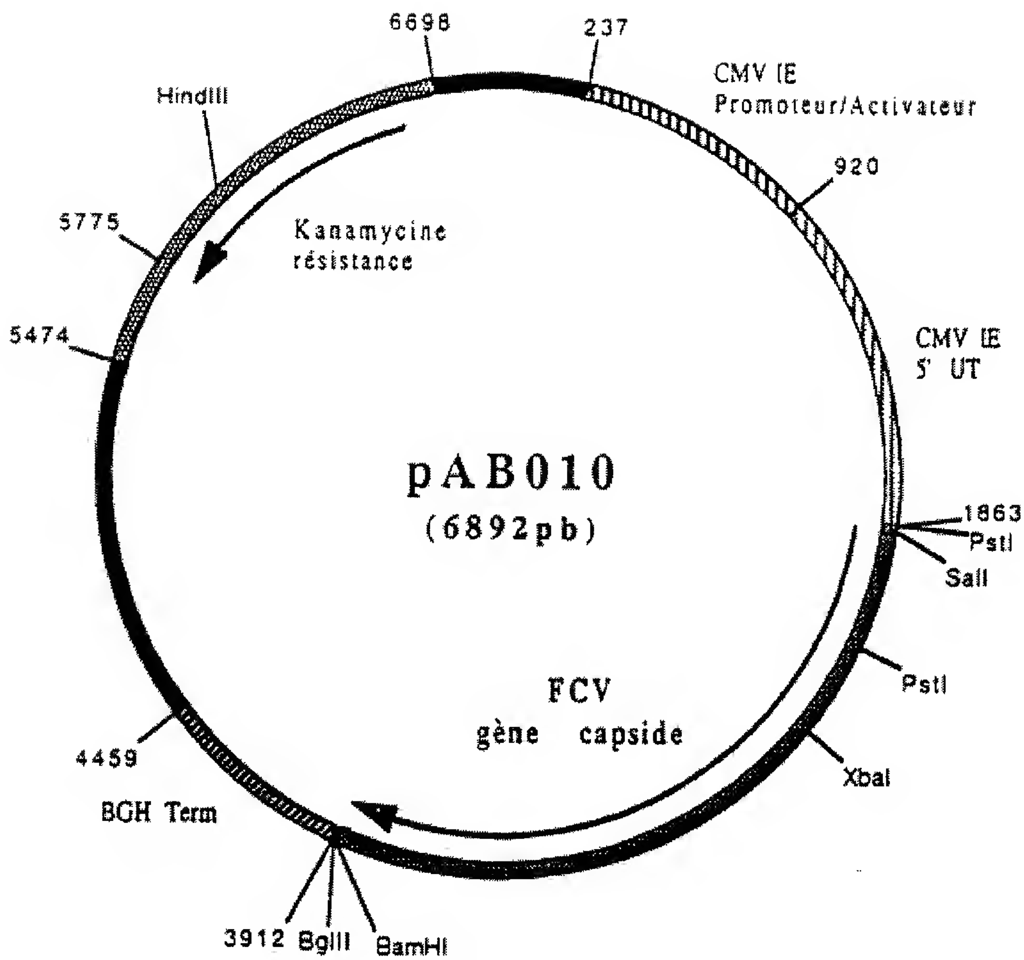

6698
237
CMV IE
Promoteur/Activateur
HindIII
920
5775
Kanamycine
résistance
5474
CMV IE
5' UT
pAB010
(6892pb)
1863
PstI
SalI
PstI
FCV
gène capside
XbaI
4459
BGH Term
3912
BglII
BamHI

Figure No. 13

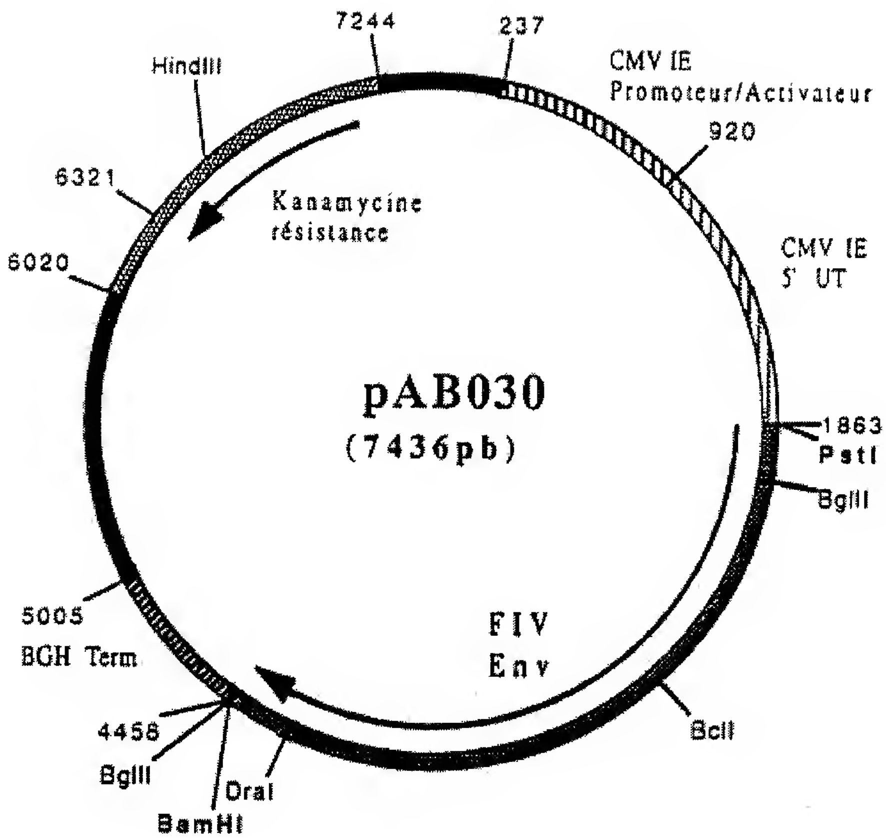

7244
237
HindIII
CMV IE
Promoteur/Activateur
920
6321
Kanamycine
résistance
6020
CMV IE
5' UT
pAB030
(7436pb)
1863
PstI
BglII
5005
BGH Term
FIV
Env
4458
BglII
BclI
DraI
BamHI

Figure No. 14

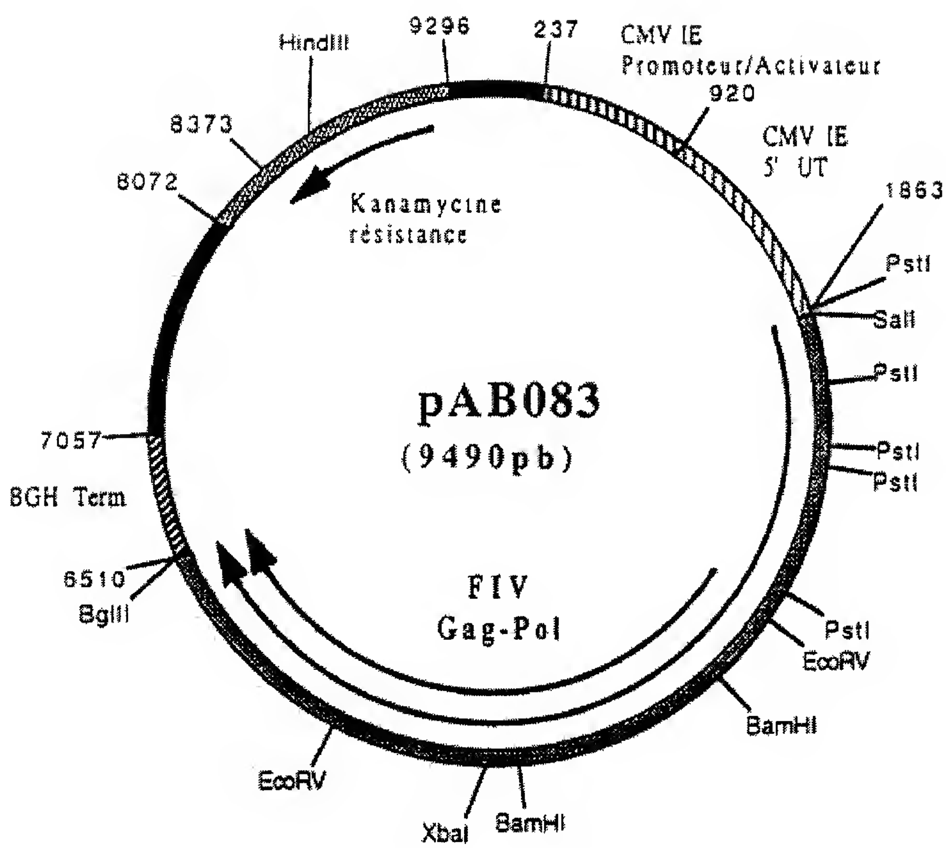
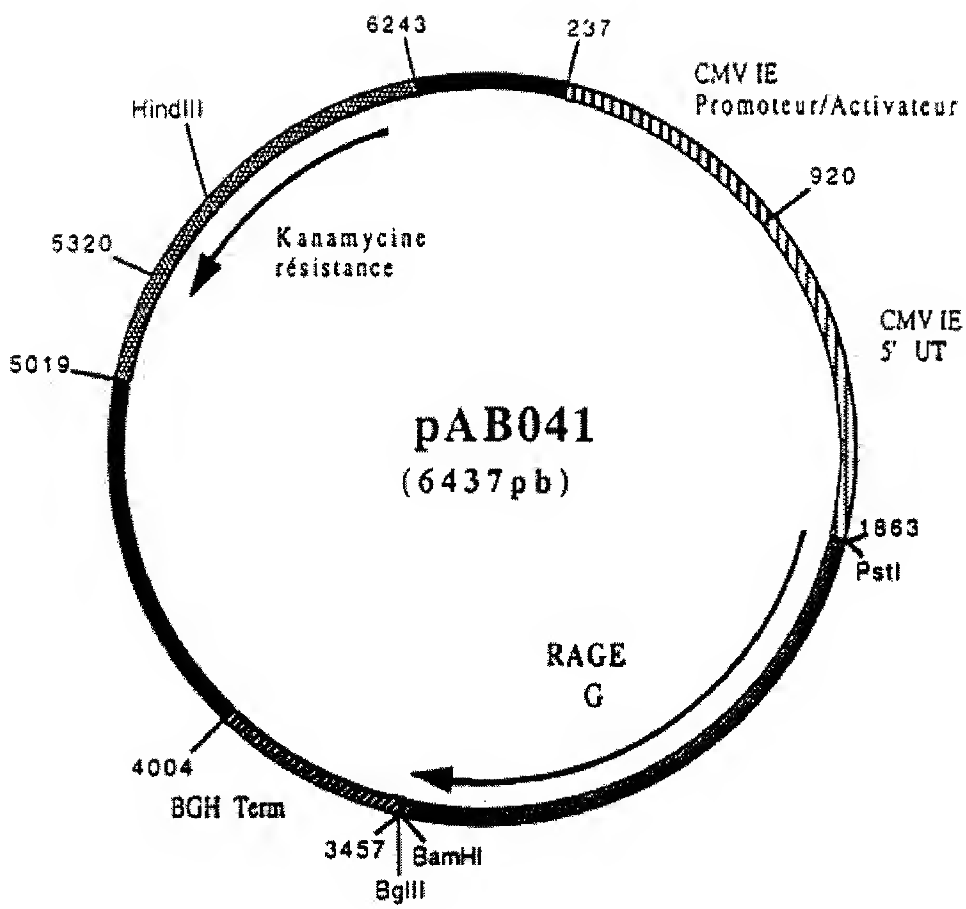

HindIII
9296
237
CMV IE
Promoteur/Activateur
920
8373
CMV IE
5' UT
8072
1863
Kanamycine
résistance
PstI
SalI
PstI
pAB083
(9490pb)
7057
PstI
PstI
BGH Term
6510
BglII
FIV
Gag-Pol
PstI
EcoRV
BamHI
EcoRV
XbaI
BamHI

Figure No. 15



6243
237
CMV IE
Promoteur/Activateur
HindIII
920
5320
Kanamycine
résistance
CMV IE
5' UT
5019
pAB041
(6437pb)
1863
PstI
RAGE
G
4004
BGH Term
3457
BamHI
BglII

Figure No. 16